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Correlation of Mixed-Function Oxidase Activity with Ultrastructural Changes in the Liver of a Marine Fish

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CORRELATION OF MIXED-FUNCTION OXIDASE ACTIVITY WITH ULTRASTRUCTURAL CHANGES IN THE LIVER OF A MARINE FISH

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Specimens of mullet (*Mugil cephalus*), a marine fish, were given single doses of 3-methylcholanthrene intraperitoneally and the activity of the microsomal mixed-function oxygenase system in the liver was measured by the metabolism of benzo(a)pyrene. The enzyme system was found to be inducible with concomitant ultrastructural changes in the hepatocytes. The specific activity and the metabolic profile approximate those of the rat.

INTRODUCTION

Enzyme induction of mixed-function oxygenase (MFO) activity in the liver of mammals has been well documented and reviewed (Conney, 1967; Remmer, 1969; Gelboin *et al.*, 1972). Although information on MFO activity in marine organisms is not as complete, the system is apparently not radically different (Pohl *et al.*, 1974; Chambers & Yarbrough, 1976). Oxidative metabolism via MFO activity has been described (Adamson, 1967) and the expoxidation of aldrin to dieldrin shown in several marine organisms (Khan *et al.*, 1972; Burns, 1976). The oxidation of polynuclear aromatic hydrocarbons such as benzo(a)pyrene [B(a)P] has also been described (Pohl *et al.*, 1974; Lee *et al.*, 1972; Pedersen *et al.*, 1974, 1976). Products of B(a)P metabolism in fish were reported to be similar to those in the rat, but excretion was apparently through urine mainly rather than feces as in the rat (Lee *et al.*, 1972). The induction of B(a)P-hydroxylase in brown trout and capelin by petroleum has been described (Payne and Penrose, 1975), but none of the metabolites were verified.

Stimulation of microsmal drug metabolism by phenobarbital (PB), chlordane, B(a)P and 3-methylcholanthrene (3-MC) in rats has been reviewed (Fouts and Rogers, 1965) and it was shown that PB and chlordane cause nonspecific, massive changes in the smooth endoplasmic reticulum (SER), whereas B(a)P and 3-MC cause specific, nonmassive changes in the SER. No changes were observed in the rough endoplasmic reticulum (RER). Changes in the SER have been found to be greater than those in the RER, and induction of cytochrome P–450 was followed by liver cell hypertrophy (Remmer, 1969).

Morphological, ultrastructural changes in the hepatocytes of mammals exposed to certain drugs and chemicals such as polycyclic aromatic hydrocarbons and other carcinogens which are capable of inducing enzymes have been reviewed (Smuckler and Arcasoy, 1969; Ghadially, 1975). The major ultrastructural change in the hepatocytes was enlargement and proliferation of the SER to varying degrees. In mammals, this proliferation has been associated with induced, enhanced detoxifying enzymes in the microsomal fraction (Remmer and Merker, 1963, 1965).

To our knowledge, little information concerning association of enzyme induction by carcinogens with ultrastructural changes in fishes has been published. Therefore, we wish to describe the formation of hydroxylated products of B(a)P, enhanced by pretreatment with 3-MC and concomitant ultrastructural changes in liver cells of mullet (*Mugil cephalus*).

METHODS

Mullet were taken by cast net from Santa Rosa Sound near Pensacola, Florida, kept in flow-through tanks and fed Jazz Surface Fish Food[R]

[R]Registered trademark, Cosby-Hodges Milling Company, 1904 North 16th. Birmingham, AL 35234. Mention of trade names or commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

once daily throughout a 7-day acclimatization and the 14-day test period. Five mullet were injected intraperitoneally with a single dose of 30 mg/kg of 3-MC (10 mg 3-MC/ml corn oil). One control and 3-MC-exposed fish each were sacrificed on days 4, 7, 11 and 14, their livers were excised for preparation of the microsomal fraction, and a portion of each liver was fixed for electron microscopy study. The liver tissue was taken directly from the freshly killed mullet, minced in 3% glutaraldehyde, and selected pieces no longer than 1 mm^3 were placed in fresh 3% glutaraldehyde for 2 h. The tissue was then postfixed in osmium, embedded in Epon^R, sectioned, and stained with uranyl acetate and lead citrate. At least three tissue specimens from different regions of each liver were fixed and sections from each examined with the electron microscope.

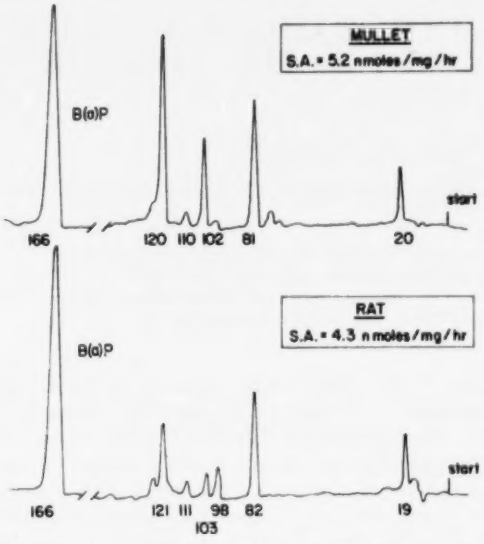

FIGURE 1 Metabolite profiles of B(a)P from liver microsomal oxidation of mullet (day 4 post-injection) and rat (day 2 post-injection) induced with a single dose of 3-MC under fasting conditions. Numbers represent relative retention. Refer to Methods section for details.

The liver microsomal fraction was isolated and the MFO activity determined in triplicate by the method of Selkirk *et al.* (1974) using 30-min incubation times. The metabolites were separated with a Micromeritics Model 7000B HPLC equipped with a Schoeffel Model 970 Fluorescence Detector (excitation: 262 nm; emission: 370 nm filter). A 25 cm by 0.4 cm stainless steel column was packed with LiChrosorb^R RP-18 (Merck) using a modified Micromeritics Column Packer at approximately 10,000 psi. Pre-purified water and methanol (Burdick and Jackson) were used in a gradient at a flow-rate of 0.6 ml/min at 50°C. All test

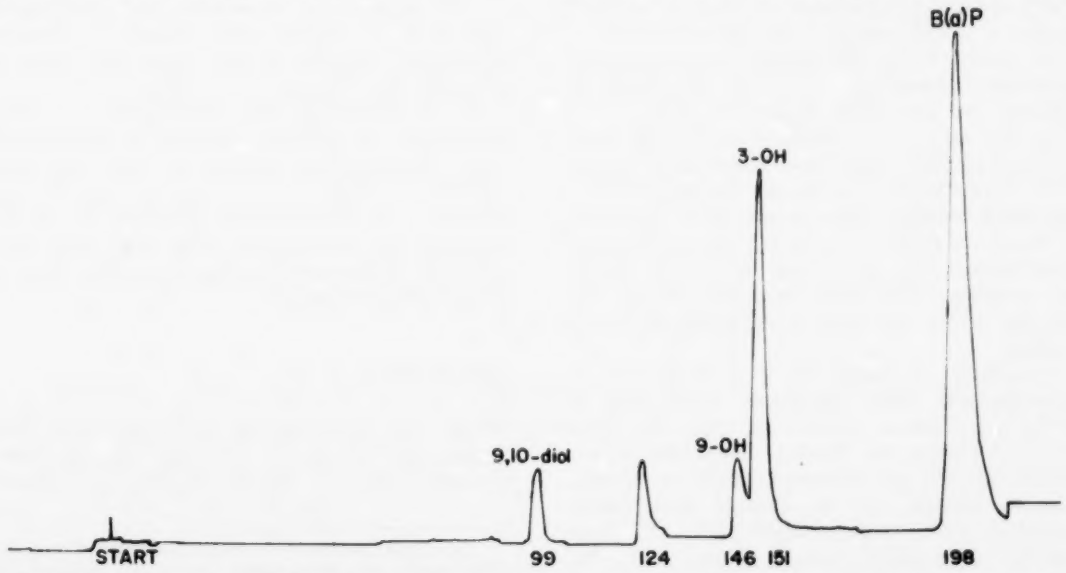

FIGURE 2 Metabolite profile from liver microsomal oxidation of mullet (day 11 exposed) induced with a single dose of 3-MC under feeding conditions.

compounds and standards were of the highest purity and obtained through the courtesy of the Illinois Institute of Technology Research Institute, Chicago.

RESULTS AND DISCUSSION

The mullet were fed during the experiment because an increase in cytochrome P-450 of 20–50% in rats during starvation has been observed (Remmer, 1969). Since involvement of cytochrome P-450 in the MFO activity is known, we hoped to circumvent the problem by feeding the animals. In addition, duration of the experiment was too long to permit fasting without causing possible significant physiological changes in the liver.

Figure 1 shows a comparison between the rat induced for 2 days and the mullet induced for 4 days in the absence of food, as determined in an earlier experiment. Direct comparison of oxygenase activities as reported by different authors is difficult because of the varied analytical methodology. It appeared to us that the method of Selkirk *et al.* (1974) as is or coupled with fluorescence detection, as in our case, would be the most accurate. Figure 2 shows the metabolite profile for an induced mullet at day 11 when fed. There is a recognizable difference in metabolite profile and enzyme activity; it is, however, of uncertain significance. Note that the early-eluting tetrols have disappeared. Note that when mullet were fed, enzyme induction did not occur until after day 4, whereas under fasting conditions, induction was found at 2 and 4 days.

MFO activity in the mullet, as measured by B(a)P metabolism, is inducible by 3-MC (Figure 3). The highest activity following induction was found at day 14 and may well have continued to rise after that. On day 11 the rate of formation of the 3-OH and 9, 10-diol accounted for the rate of disappearance of about 30% of B(a)P, the parent compound. Other metabolites were present but could not be positively identified. The identity of the B(a)P and of the 3-OH, 9-OH, and 9, 10-diol metabolites was determined by comparison to relative retentions of the pure compounds. Confirmation was achieved by stopping the solvent flow at the top of the peak, obtaining excitation and emission spectra, and comparing the spectra to those of standards.

Ultrastructural changes were found in liver tissue samples taken on days 4, 7 and 11 post-injection.

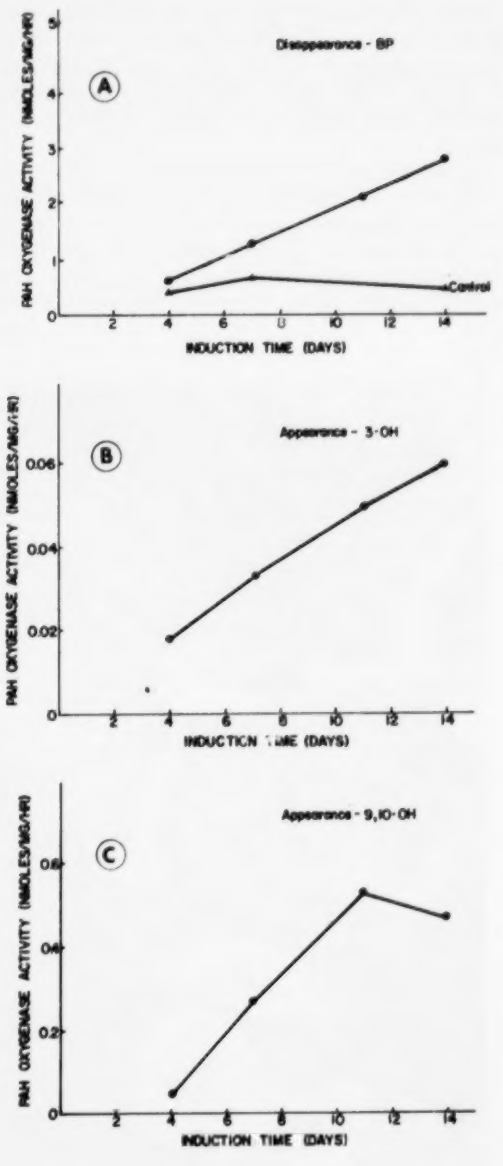

FIGURE 3 Specific activity of mixed-function oxygenase based on (a) the disappearance of B(a)P: control *vs* exposed, (b) the appearance of the 3-OH derivative and (c) the appearance of the 9,10-diol derivative. All points are averages of three deerminations with a maximal error of ±5%.

Certain of these changes can be correlated with increase enzyme activity (Figure 3), and other changes appear to reflect stress and degenerated conditions in the mullet hepatocyte that probably resulted from cellular intoxication following injections.

Figure 4 shows hepatocytes of control fish at day 7 which are interpreted as normal because of the following characteristics: extensive lakes of glycogen in cytoplasm, limited perinuclear cytoplasm containing normal RER and mitochondria, and normal nuclei with expected distribution of heterochromatin and normal nucleolar morphology. The following hepatocyte changes were found in different post-injection period liver samples: day 4 exposed fish hepatocytes had loss of most cytoplasmic glycogen reserves; day 7 exposed fish revealed several hepatocyte changes including (a) loss of glycogen (Figure 5), (b) proliferation of RER and SER (Figure 6), (c) enlargement of ER vesicles (Figures 6, 7) and (d) appearance of some dilated SER (Figures 6, 7); day 11 exposed fish hepatocytes appeared to have advanced degenerative changes resembling those in hepatocytes of day

FIGURE 4 Hepatocyte profiles from control, normal fish (*Mugil cephalus*): note large glycogen reserves, normal nuclei, modest penirucular cytoplasm with rough endoplasmic reticulum (RER) and normal mitochondria. Blood sinus contains nucleated red blood cells and is surrounded by space of Disse. × 11,376.

7 exposed fish with the additional changes of mitochondrial swelling, pigment deposition and early intermediate nuclear degeneration characterized by nucleolar loss or condensation (Figure 8) and chromatin margination (Figure 8) suggesting early necrosis. The hepatocytic changes in the day 7 and day 11 exposed fish consisting of proliferation and enlargement of ER and appearance of dilated SER may be correlated with the increased enzymatic activity found at day 7 (Figure 3).

We have demonstrated the ability of mullet to hydroxylate B(a)P, that this activity is inducible by 3-MC, and that the metabolite profile is similar to that found in rats. The proliferation and enlargement of the ER strongly suggests that the mullet hepatycotes behave similarly to other vertebrate hepatocytes when exposed to enzyme-inducing chemicals. The nuclear changes in the hepatocytes of the injected fish reflect cellular necrosis due to cellular intoxication. In spite of feeding, early loss of liver glycogen may be indicative of nutritional as well as other stress such as confinement.

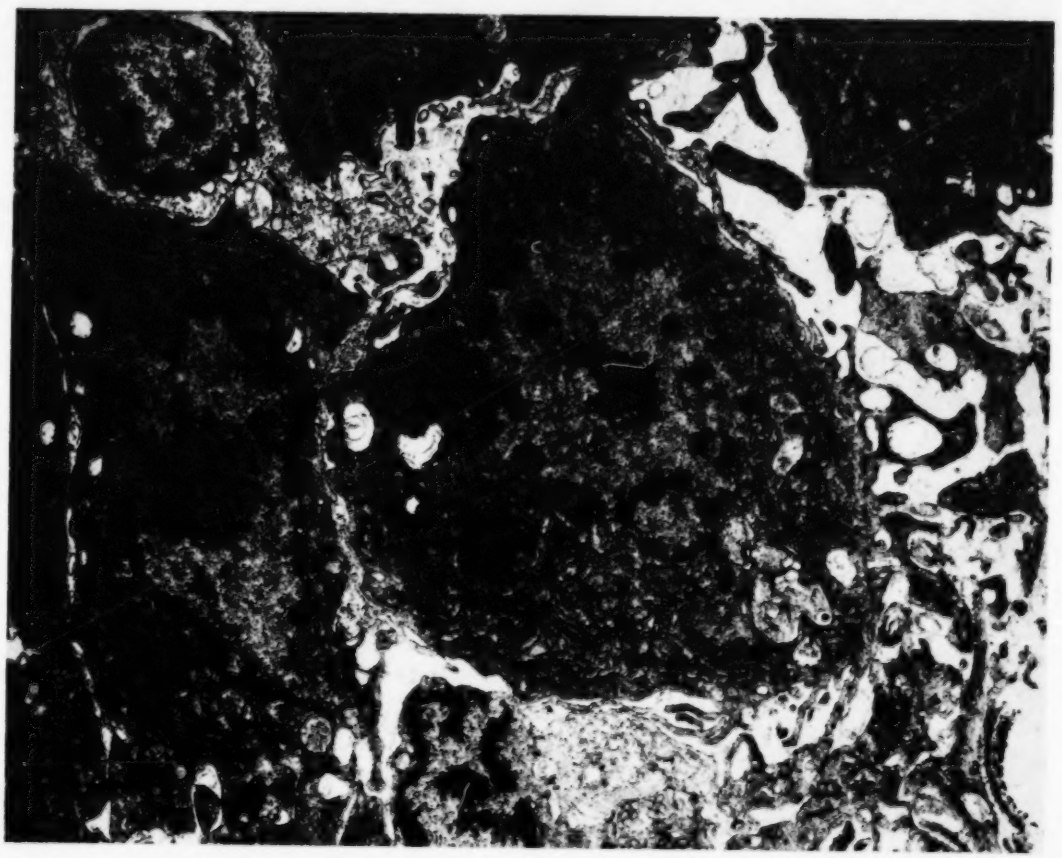

FIGURE 5 Profiles of hepatocytes from day 7 exposed fish; compare with normal hepatocyte profiles in Figure 4. Note the loss of glycogen, increased RER. ×14,400.

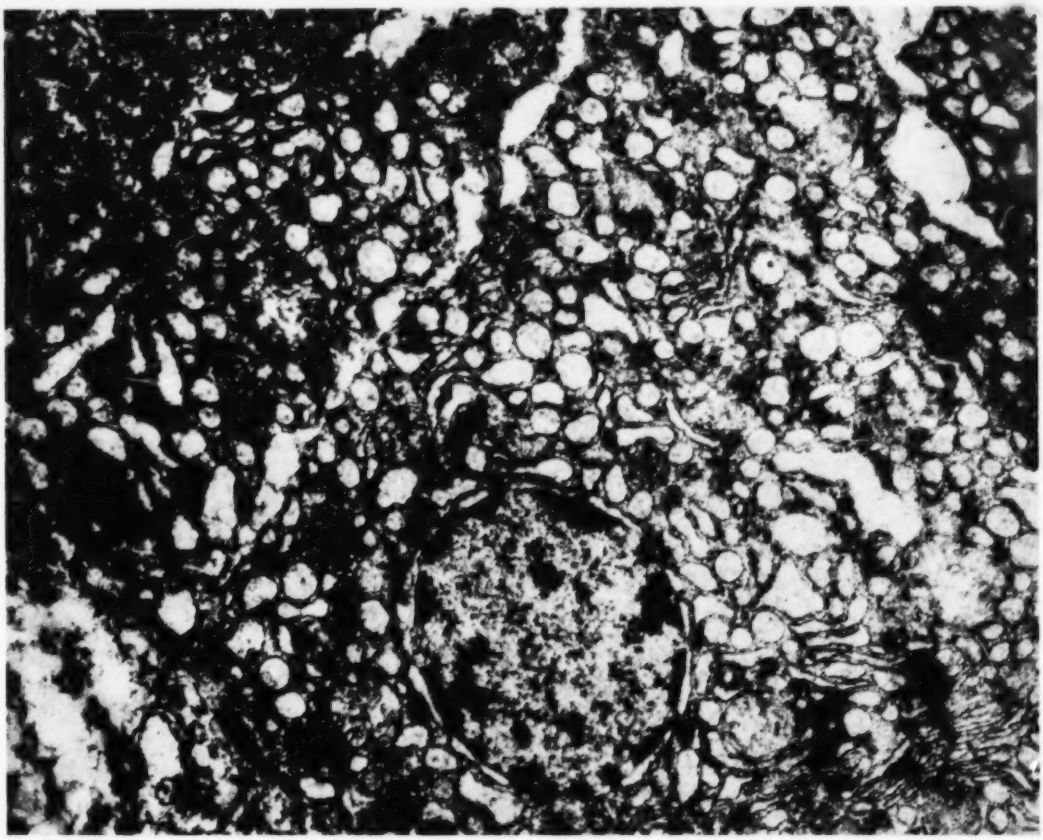

FIGURE 6 Profile of hepacocyte from day 7 exposed fish; note the increased profiles of both RER and SER and dilation of ER vesicles. ×14,400.

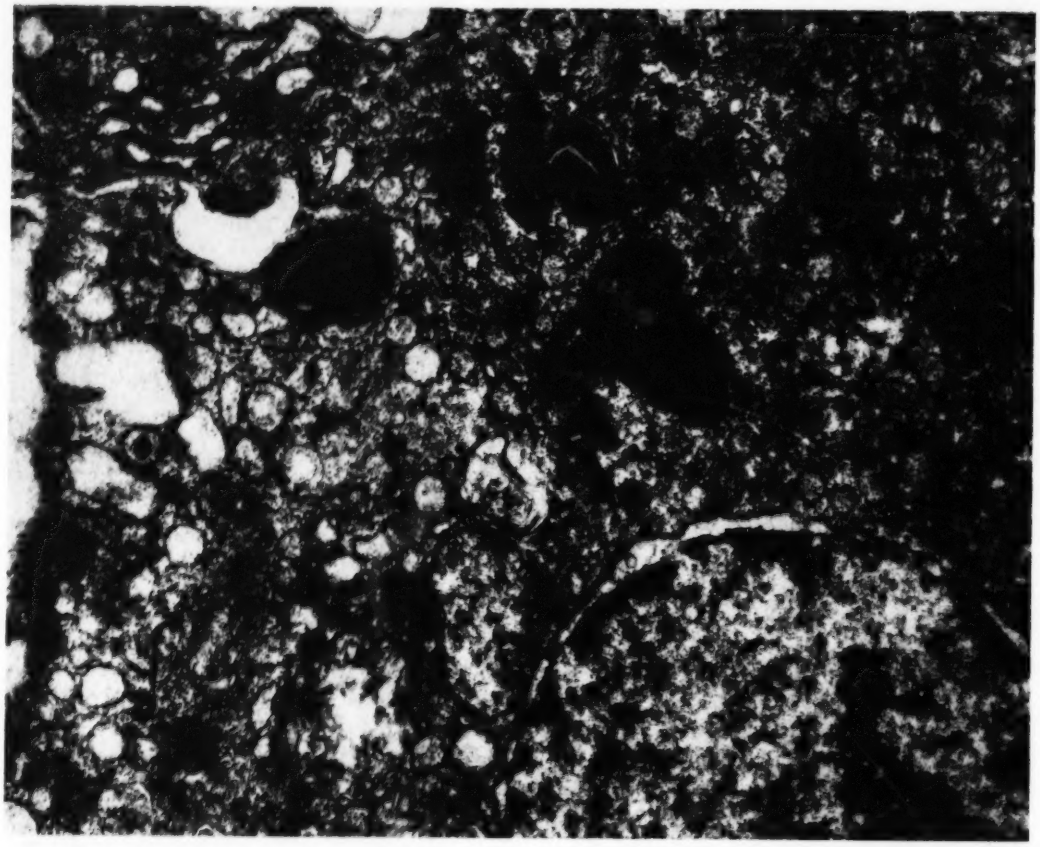

FIGURE 7 Early deposition of electron opaque substances, proliferation and swelling of RER and SER in day 7 exposed fish hepatocytes. ×28,500.

FIGURE 8 Day 11 exposed fish hepacocyte profiles; note swollen mitochondria, pigment deposition, and nuclear changes including chromatin margination. Cytoplasmicdensity and cell boundaries are altered compared to normal hepatocytes (Figure 4). ×9,519.

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